

# EPA Chemomorphic Analysis of Malathion in Skin Layers: Implications for the Use of Dermatopharmacokinetic (DPK) Tape Stripping In Exposure Assessment to Pesticides

C C Dary<sup>1</sup>, and M A Saleh <sup>2</sup>. <sup>1</sup>U.S. EPA, Human Exposure and Atmospheric Sciences Division, Las Vegas, NV; <sup>2</sup>Environmental Chemistry and Toxicology, Texas Southern University, Houston, TX

## Abstract

The dermatopharmacokinetic (DPK) method of dermal tape stripping may prove to be a valuable addition to risk assessment protocols for toxic substances. To examine this possibility, the dermal penetration and absorption characteristics of [<sup>14</sup>C]-malathion in the Sprague-Dawley rat were examined by three analytical techniques. [<sup>14</sup>C]-malathion was applied in different vehicles for 30-minute and one-hour periods of exposure. Penetration into the stratum corneum (SC) was assessed by tape stripping followed by Instant Electronic Autoradiography (IEA). Also, the [<sup>14</sup>C]-activity retained in three successive 16  $\mu$ m sections of the skin application site was determined by IEA and malathion was identified by Fourier Transform Infrared Microscopy (FTIR microscopy). Absorbed [<sup>14</sup>C]-malathion was measured in selected tissues, organs, and the residual carcass by Liquid Scintillation Counting (LSC). Penetration into the SC followed a linear trend. The capacity of the SC reservoir for malathion amounted to approximately 1% of the dermal dose, while approximately 6% of the dose was absorbed. Results from this study support the view that LSC remains the method of choice to efficiently and reliably quantify absorption of a radiolabeled test substance. IEA offers the ability of the user to visualize the extent and profile of dermal absorption. When IEA is combined with FTIR microscopy, an effectual tool for studying the penetration of chemicals into layers of the skin emerges. The combined use of the three analytical techniques can be used to test the validity of the DPK method in hazard evaluation and exposure assessment of the organophosphorus insecticides.

The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development, participated in this research and approved this abstract as a basis for a poster presentation. The actual presentation has not been peer reviewed by the EPA.

## Methods

### Animals and Housing

Adult male albino rats (Harlan Sprague Dawley Inc., Indianapolis, Indiana)

Environmental controls: Air temperature 21°C  $\pm$  1° and relative humidity of 50%  $\pm$  10% under a 12-hour light/dark cycle.

### Test Materials

Technical grade neat malathion (purity 94.6%) obtained from American Cyanamid, Princeton, New Jersey.

Commercial product, 50% emulsifiable concentrate (EC), obtained from Southern Mill Creek Products, Inc., Houston, Texas.

Radiolabeled [<sup>14</sup>C]-malathion (14.2 mCi/mmol) purchased from Sigma Chemical Company, St. Louis, Missouri.

### Preparation of dosing solutions

Technical grade [<sup>14</sup>C]-malathion: (1.12 g) dissolved in 200  $\mu$ L of [<sup>14</sup>C]-malathion stock solution.

Dosing solution of the 50% commercial product: (2.24 g) of the EC concentrate mixed with 200  $\mu$ L of [<sup>14</sup>C]-malathion stock solution.

Aqueous dosing solutions: two 100  $\mu$ L aliquots of the [<sup>14</sup>C]-malathion stock solution (5.94  $\mu$ Ci) allowed to evaporate to near dryness before mixing the residual [<sup>14</sup>C]-malathion with 100 mL tap water and the appropriate mass of 50% commercial product to produce 1% (1.02 mg) and 10% (10.2 mg) aqueous mixtures.

A similar procedure was followed to prepare non-radiolabeled dosing solutions for use in the FT-IR analysis of dermal penetration. Technical grade malathion was prepared by dissolving 5.10 grams of neat malathion in 10.0 mL absolute ethanol. Similarly, a dosing solution of the 50% EC formulation was prepared by mixing 10.2 grams of the EC concentrate in 10.0 mL absolute ethanol.

### Experimental Designs

The [<sup>14</sup>C] labeled neat malathion and 50% commercial product were used in an abridged *in vivo* dermal tissue distribution study (Experiment 1) as specified under U.S. EPA Office of Prevention, Pesticides, and Toxic Substances (OPPTS) Health Effects Test guidelines 870.7600 (U.S. EPA, 1998). Animals were assigned to groups according to the vehicle, source of the dosing solution and duration of exposure.

### Experimental procedures

Following a 24-hour period of acclimation, a 10 cm<sup>2</sup> area of skin on the dorsal region of the shoulders and back of each animal was clipped. Care was taken to avoid abrading the skin. The clipped area was washed with ethanol prior to dosing. The assigned dose in each experiment was applied evenly to the site of application (clipped skin area). A non-occlusive cover was used to prevent mechanical loss of test material (Dary et al., 1994). After dosing, the animals were maintained individually in metabolism cages. At the end of each assigned experimental period, the non-occlusive covers were carefully removed and kept for radiochemical analysis.

### Sample collection

At the time of sacrifice, the following tissues were obtained for radiochemical analysis: brain, heart, lungs, liver, kidneys, small intestine, large intestine, stomach, testes, skin at the site of application, and the remaining carcass. The skin of the application site was divided into four equal surface sections. One portion was stripped of layers of stratum corneum by eight successive strippings (application and removal) with adhesive tape. Another portion was subjected to Liquid Scintillation Counting (LSC). The other portions were sectioned and examined for radioactivity using Instant Electronic Autoradiography (IEA).

### Analysis

Radioactivity in different tissues as well as non-occlusive covers was measured by Liquid Scintillation Counting (LSC) using a Wallac 1409 Liquid Scintillation Counter. A known weight of the homogenized tissue was oxidized using a Packard model 307 automatic sample oxidizer (Packard Instruments Company, Meriden, CT). All samples were prepared in duplicates.

Autoradiography was performed on successive adhesive tape strips and frozen planar thin sections (16  $\mu$ m) of skin excised from the site of application. The frozen sections were mounted on the lower third of the frosted end of pre-cleaned microscope slides. The sections were scanned using an Electronic Autoradiography Instant Imager (Packard Instruments Company, Meriden, CT) for an acquisition time of 2 hours. Radiomicrographs were made of certain sections.

The application sites were sectioned into three successive 16  $\mu$ m planar sections each using a freezing microtome (Minotome, International Equipment Company). The sections were mounted on 3M Type 61 disposable IR cards (5 cm X 10 cm) and then freeze dried. The FTIR microscopy was performed on a Perkin Elmer instrument, FTIR Microscope System 2000.

## Results

**Table 1.** Recovery of malathion in eight successive tape strips as determined by electronic autoradiography.

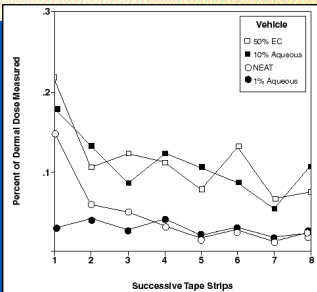
Group	Vehicle	Source	Exposure Duration (hrs)	Number of Animals	[ <sup>14</sup> C]-malathion eqva val ents (#g)	Dose recovered (%)
A	ETOH	NEAT	0.5	3	<b>93.9 <math>\pm</math> 19.9</b>	<b>0.84 <math>\pm</math> 0.18a</b>
B	ETOH	NEAT	1.0	3	<b>35.5 <math>\pm</math> 25.4</b>	<b>0.32 <math>\pm</math> 0.30a</b>
C	ETOH	50% EC	0.5	3	<b>28.3 <math>\pm</math> 18.3</b>	<b>0.25 <math>\pm</math> 0.16a</b>
D	ETOH	50% EC	1.0	3	<b>14.2 <math>\pm</math> 7.8</b>	<b>0.13 <math>\pm</math> 0.07a</b>
E	10% ETOH	50% EC	0.5	3	<b>167 <math>\pm</math> 76</b>	<b>1.47 <math>\pm</math> 0.68a</b>
F	10% ETOH	50% EC	1.0	3	<b>563 <math>\pm</math> 79</b>	<b>1.88 <math>\pm</math> 0.71a</b>

a, b = means ranks for group are homogeneous  $\alpha$  = 0.05

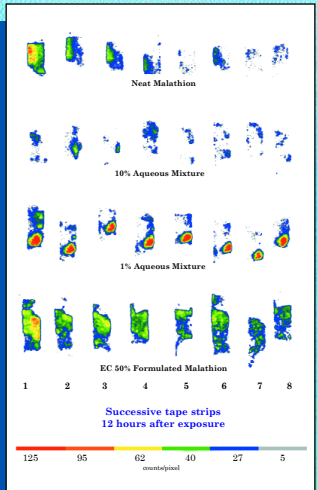
**Table 2.** Measurements of [<sup>14</sup>C]-malathion equivalents in eight successive adhesive tape strips taken from the site of application as determined by electronic autoradiography.

Group	Mean (µg) and (percent of dermal dose)								Total
	1	2	3	4	5	6	7	8	
G - Neat	153 $\pm$ 3	59.5 $\pm$ 4.2	50.4 $\pm$ 3.1	33.8 $\pm$ 2.4	16.5 $\pm$ 2.3	27.9 $\pm$ 1.3	13.4 $\pm$ 4.3	22.0 $\pm$ 1.1	<b>375 <math>\pm</math> 18</b>
	(0.15)	(0.06)	(0.05)	(0.03)	(0.02)	(0.03)	(0.01)	(0.02)	(0.37)
H - 50% EC formulation	222 $\pm$ 7	108 $\pm$ 2	126 $\pm$ 2	114 $\pm$ 4	76.0 $\pm$ 2.2	133 $\pm$ 2	66.3 $\pm$ 10.5	74.7 $\pm$ 1.0	<b>923 <math>\pm</math> 23</b>
	(0.22)	(0.11)	(0.12)	(0.11)	(0.08)	(0.13)	(0.06)	(0.07)	(0.86)
I - 10% Aqueous mixture	18.3 $\pm$ 0.5	13.6 $\pm$ 3.7	8.72 $\pm$ 0.1	12.6 $\pm$ 6.6	10.8 $\pm$ 0.3	8.69 $\pm$ 0.3	5.4 $\pm$ 1.1	10.8 $\pm$ 0.3	<b>86.5 <math>\pm</math> 3.4</b>
	(0.18)	(0.13)	(0.09)	(0.12)	(0.11)	(0.09)	(0.05)	(0.11)	(0.85)
J - 1% Aqueous mixture	0.28 $\pm$ 0.02	0.41 $\pm$ 0.02	0.27 $\pm$ 0.14	0.41 $\pm$ 0.02	0.20 $\pm$ 0.02	0.30 $\pm$ 0.01	0.17 $\pm$ 0.10	0.22 $\pm$ 0.02	<b>2.37 <math>\pm</math> 0.27</b>
	(0.03)	(0.04)	(0.03)	(0.04)	(0.02)	(0.03)	(0.02)	(0.02)	(0.22)

The percent of the dose recovered for each of the dosing solutions declined with each successive tape strip. The decline in the percent of the dose recovered followed a linear trend as determined by the test of trend of the successive series of tape strips (Figure 1).



**Figure 1.** Decline in the percent of the dose in successive tape strips of the site of application of neat malathion, the 50% EC formulation, 10% aqueous mixture and 1% aqueous mixture as determined by electronic autoradiography.



**Figure 2.** <sup>14</sup>C Activity measured in successive tape strips of the skin of the application site following 1 hour of exposure to neat malathion, the 50% EC formulation, and the 10% and 1% aqueous mixture as determined by electronic autoradiography.

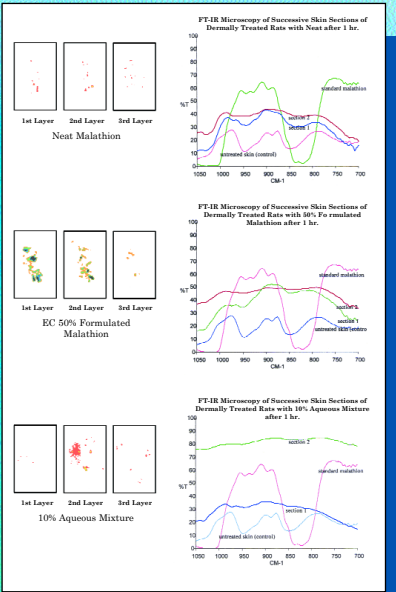
As might be interpreted from the appearance of the profiles, a significant interaction ( $F_{21,56} = 27.567$ ,  $p < .001$ ) was found between the repeated measures for the tape strips and the four dosing solutions. The mean percent of the dose for the 50% EC formulation recovered in the first tape strip (0.22%) was significantly greater than the 10% aqueous mixture (0.18%), the neat malathion (0.15%), and the 1% aqueous mixture (0.03%).

The electronic autoradiographs of the eight successive tape strips depicts recovery of [<sup>14</sup>C]-malathion for each of the four dosing solutions following one hour of exposure (Figure 2).

The intensity of the images correspond with the measurement of <sup>14</sup>C activity. The maximum activity in the successive tape strips was observed for the EC 50% commercial formulation.

The autoradiographs of the three serial sections confirmed the spotty nature of the localization of the <sup>14</sup>C activity (Figure 3).

The highest amount of <sup>14</sup>C activity was detected in the first section of the skin treated with the 50% EC commercial formulation. The results also indicated that most of the <sup>14</sup>C activity was retained in the external surface of the skin. Fourier Transform Infrared Microscopy confirmed the nature of the <sup>14</sup>C activity as [<sup>14</sup>C]-malathion in three serial sections of the skin of the application sites for the 10% aqueous mixture, the 50% EC formulation and neat malathion. The FTIR spectra showed two absorption bands at 823 cm<sup>-1</sup> and 835 cm<sup>-1</sup> which are characteristic of the presence of the P=S group of malathion.



**Figure 3.** <sup>14</sup>C Activity measured in three successive (16  $\mu$ m) thin sections of the skin of the application site exposed to neat malathion, the 50% EC formulation and 10% aqueous mixture as detected by FTIR microscopy.

## Discussion

Penetration has been defined by Schaefer et al. (1978) as the entrance process into a single layer while permeation refers to the migration through one or several skin layers and absorption is the sum of penetration, permeation and "release" into cutaneous blood and lymph tissues. Penetration of a test substance into the stratum corneum (SC) can be determined by successive removal of the SC by repeated application of adhesive tape strips followed by "stripping" of the skin surface (Fredriksson, 1961; Schaefer et al., 1978; Dupuis et al., 1986; Rougier et al., 1983, 1987). The amount of penetrant recovered decreases with each successive tape strip corresponding with the depth of penetration in a logarithmic fashion (Schaefer et al., 1978; Tsai et al., 1991).

This apparent curvilinear pattern of recovery was observed with malathion (Figure 1) for the neat malathion, the 50% EC formulation and 10% aqueous dosing solutions but was not evident for the 1% aqueous dosing solution. This apparent logarithmic pattern was satisfied by a significant linear trend. The slopes of the penetration profiles were dependent on the vehicle and source of malathion (neat or 50% EC formulation) as indicated by the significant interaction between the repeated tape strippings and the four dosing solutions. This significant interaction, as explained by Schaefer et al. (1978), is not likely to be strictly a result of more efficient or complete stripping (Tsai et al., 1991), although it has been amply demonstrated that the amount of SC material (and therefore penetrant) removed with each successive tape strip decreases with increasing depth (Schaefer et al., 1978).

The efficiency of recovery of <sup>14</sup>C activity that possibly coincides with tissue removal and depth of penetration may be pictorially assessed from the electronic autoradiograms of the tape strips for the four dosing solutions (Figure 2). The autoradiograms clearly show the spotty nature of the recovery and the decline in the recovery with each successive tape strip. The spotty accumulation of <sup>14</sup>C activity in the three 16  $\mu$ m serial sections of the application site (Figure 3) was confirmed as [<sup>14</sup>C]-malathion by FTIR microscopy which successfully demonstrated the disposition of malathion in the skin regions. This logarithmic pattern is evident for a broad range of compounds with diverse physicochemical properties and may represent a factor governing percutaneous absorption (Rougier, 1990). According to Rougier (1990), the total mass of a substance recovered in the successive tape strips represents the capacity of the SC reservoir for that substance. Rougier (1990) offered that measurement of a chemical in the SC by tape stripping at the end of a period of exposure, e.g., 30 minutes, would give a good assessment of absorption within a specified sampling period, e.g., 4 days.

## Conclusion

Certainly, more work like that of Rougier (1990) is needed with pesticides before the predictive value of tape stripping is realized for dermal exposure assessment. Indeed, the relevance of what has been termed the dermatopharmacokinetic (DPK) method of skin tape stripping (U.S. FDA, 1998) to clinical efficacy and safety has been under review and debate (Pharmaceutical Research and Manufacturers of America, 1998). Before the DPK method can find application in hazard evaluation and exposure assessment, the stripping technique must be validated and verified for toxic substances of interest to EPA in a manner consistent with protocols established for topical drugs (Rougier and Lotte, 1993; Shah et al., 1998). Testing requirements must be established for the preparation of the test system, selection of test and control articles and vehicles in accordance with the analytical technique, radiometric (IEA or LSC) or spectrophotometric (FT-IR, AMS, or gas or liquid chromatography), setting the exposure duration, e.g., 0.5 hours, and sample collection period, e.g., 96 hours (Rougier and Lotte, 1993), application of the tape stripping method (number of strips), and expression of results, e.g., mass of test article/mass of tissue. Once these testing requirements are established, relationships must be found between the SC reservoir capacity at a specified exposure duration and the amount of test substance absorbed over a specified sampling period for a group of compounds with a range of physicochemical properties. Verification of the valid use of the DPK method in hazard evaluation would legitimize the effective use of the method in exposure assessment where invasive techniques with human subjects are problematic.

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